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SCIENTIFIC PROCEEDINGS

OF THE

ROYAL DUBLIN SOCIETY.

VOL. 19 (N.S.).

[SEPARATE ISSUE.]

No. 31. FEBRUARY, 1930.

A STUDY OF TWO NEW SPECIES OF BACTERIA BELONGING TO THE GENUS *CHROMOBACTERIUM*.

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Price Sixpence.

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A STUDY OF TWO NEW SPECIES OF BACTERIA BELONGING TO THE GENUS *CHROMOBACTERIUM*.

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[Read November 26, 1929. Published separately February 8, 1930.]

In the course of the bacteriological examination of thirty-six samples of water, bacteria belonging to the genus *Chromobacterium* were isolated in seventeen cases. These seventeen samples of water were taken, either from surface wells, or from deep wells that the bacteriological examination showed to be contaminated by the admixture of surface waters. The waters were plated in duplicate in dilutions of 1 c.c., 1/10 c.c., and 1/100 c.c., using lactose nutrient agar, and the Petri dishes incubated at 37° C. for two days, and at 21° C. for seven days.

The number of colonies of bacteria belonging to the genus *Chromobacterium* that were found on the Petri dishes incubated at 21° C. for a week was generally less than 10 per c.c., and usually accompanied by bacteria belonging to the genus *Pseudomonas*. Bacteria belonging to the genus *Chromobacterium* were never found on the corresponding Petri dishes incubated at 37° C.

Sixteen of the seventeen cultures examined resembled one another so closely that they were considered to be the same species and are represented by V 1. The sixteen cultures closely resembled one another morphologically, but differed slightly in the rate of development of the violet pigment, in the rate of liquefaction of gelatine, and in the type of digestion of sterile milk, but these differences were not considered marked enough for them to be regarded as different species. This type occurs in many parts of Ireland, having been isolated from samples of water received for bacteriological examination from County Cork, County Limerick, County Tipperary, and County Galway, and it has been named *Chromobacterium hibernicum.*

The culture known as V 3 was isolated from a sample of water drawn from a newly bored artesian well, sunk to a depth of 66 feet. The total bacterial count at 21° C. was 5,200 per c.c., and only the one colony was found. The growth form of this bacterium is characterized by its dark violet sheen and its tenacity. It tends to come away from the medium on which it is grown as a film, and the growth can be lifted off the medium in large patches by inserting

a platinum needle under it. In consequence, it has been named *Chromobacterium cohaerens.*

Both species of bacteria reduce nitrates, whereas all the species listed in Bergey's "Manual of Determinative Bacteriology" are stated not to reduce nitrates. *Chromobacterium cohaerens* has a positive diastatic action, whereas *Chromobacterium hibernicum* is negative.

V 1.

Found in water from surface wells.

Morphology.

Slender rods 1·0μ to 1·75μ by 0·5μ to 0·75μ, aerobic, motile, non-spore-forming, gram-negative, may be gram-variable.

Cultural Characters at 21° *C.*

Nutrient Agar.—Seven days—surface colonies—may be circular 1 to 6 mm. diameter, or irregularly shaped 3 to 8 mm. by 2 to 4 mm.—grayish at first, violet pigment starts to develop in from 2 to 4 days at edge of colonies and spreads inwards towards centre. Colonies glistening, raised, opaque, finely granular; edge definite, entire. Sub-surface colonies 1 to 2 mm., oval, do not develop pigment for several days.

Nutrient Agar slant. — Seven days—growth good, grayish at first, turning violet at the edges, violet pigment spreading inwards towards the centre. Growth glistening, smooth, raised, opaque; definite edge, entire or undulate.

Nutrient agar stab.—Seven days—good growth, violet pigment on surface. Growth grayish along line of stab.

Nutrient gelatine.—Five days—slight to fair liquefaction, colonies circular, granular, grayish colour at first, turning violet, edge entire.

Nutrient gelatine stab.—Ten days—gelatine one-sixth liquefied, stratiform, filiform growth along line of stab, violet pigmented growth in the liquefied gelatine. Gelatine two-thirds liquefied in from three to six weeks.

Potato slope.—Seven days—good growth, colour at first brownish, finally dark violet, glistening, raised, rugose, opaque, definite edge.

Physiological Characters at 21° *C.*

Nutrient broth.—Seven days—growth, cloudiness, violet pigmented film on surface, and violet coloured sediment; pigment diffused through liquid.

Dunham's solution.—Seven days—growth, cloudiness, violet pigmented film on surface, violet pigmented deposit at bottom of test-tube.

Fermentation.—Ten days—neither acid nor gas formed in galactose, glycerol, glucose, inulin, lactose, levulose, maltose, mannitol, salicin, and saccharose broths. Development of violet pigment on surface of sugar broths.

Plain milk. — Two to four days—growth, violet pigment on surface, violet colour diffused through upper layer of milk. Ten days—upper part of milk of a dense dark violet colour, with dark violet film on surface. Violet colour diffused through milk and dark violet sediment. Soft coagulation and partial digestion of milk in from four to six weeks. Slight increase in acidity from 0·17 to 0·20 per cent. lactic acid. Total count (five days) = 400,000,000 per c.c.

Litmus milk. — A slight development of acidity, with the same changes taking place as described under "Plain milk."

Potato utilization as indicated by hydrogen peroxide (H_2O_2) (Gull, Jean, and Broadhurst). H_2O_2 positive.

Diastatic action—negative.

Indol—negative.

Nitrates—reduced.

Remarks—growth scanty or absent at 37° C.

The name suggested for this bacterium is *Chromobacterium hibernicum.*

V 3.

Found in water obtained from a recently sunk artesian well, 66 feet deep.

Morphology.

Rods 1·25μ to 3·0μ by 0·75μ to 1·0μ, aerobic, non-spore-forming, motile, generally gram-negative, may be gram-variable, may form chains (especially noticeable when grown in gelatine media).

Cultural Characters at 21° *C.*

Nutrient agar. — Four days—colonies 2 to 5 mm. diameter, dark violet, glistening, raised, butyrous, opaque, finely granular, generally circular, edge entire or slightly indefinite. Sub-surface colonies, oval 1 to 2 mm. long, do not develop pigment for several days.

Nutrient agar slant. — Seven days—growth good, dark violet, glistening, smooth to rugose, opaque, raised, edge entire. The growth is characterized by its tenacity, the growth can be lifted off media in large patches by inserting needle under it.

Nutrient agar stab.—Seven days—good grayish growth along line of stab, grayish to dark violet pigment on surface.

Nutrient gelatine.—Five days—marked liquefaction—colonies sunk—diameter 1 to 3 mm., granular, opaque, grayish at first, finally violet, edge entire or slightly irregular.

Nutrient gelatine stab.—Ten days—gelatine one-quarter to one-half liquefied, saccate, filiform grayish growth along line of stab. Growth in liquid grayish to violet. The development of violet pigment is not as marked as in *Chromobacterium hibernicum.*

Potato slope.—Seven days—growth poor to fair, glistening, raised, opaque, rugose, dark violet, definite edge.

Physiological Characters at 21° C.

Nutrient broth.—Seven days—growth, cloudiness, grayish film on surface turning violet, grayish violet sediment.

Dunham's solution.—Seven days—growth, cloudiness, gradual development of violet pigment on surface and violet pigmented deposit at bottom of test-tube.

Fermentation.—Ten days—neither acid nor gas formed in galactose, glycerol, glucose, inulin, lactose, levulose, maltose, mannitol, salicin, and saccharose broths.

Plain milk.—No evident change for from twenty-four to forty-eight hours, then slight grayish to bluish violet pigment develops at surface, and in ten days coagulation with soft curd, and development of violet pigment at surface of milk. Partial digestion of milk in from two to four weeks. Total count (five days) = 300,000,000 per c.c.

Litmus milk.—No marked development of either acidity or alkalinity, the same changes taking place as described under "Plain milk."

Potato utilization as indicated by hydrogen peroxide (H_2O_2) (Gull, Jean, and Broadhurst). H_2O_2 positive.

Diastatic action—positive.

Indol—negative.

Nitrates—reduced.

Remarks—Growth scanty or none at 37° C.

The name suggested for this bacterium is *Chromobacterium cohaerens.*

Grateful acknowledgment is made of the kindness of Dr. R. St. John-Brooks, Curator, National Collection of Type Cultures, Lister Institute of Preventive Medicine, London, in confirming the reduction of nitrates by *Chromobacterium hibernicum* and *Chromobacterium cohaerens,* and in forwarding type cultures for comparison. Acknowledgment is also made of the kindness of Dr. G. H. Weaver, Curator, American Type Culture Collection, Chicago, U.S.A., in forwarding a type culture for comparison.